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Detection of *Escherichia coli* O157 in vegetables

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Abstract: *Vegetables can be vehicles for transmitting E. coli O157 to humans; therefore this study was carried out in order to investigate the presence of this strain of Enterohemorrhagic E.coli in different kinds of vegetables as well as vegetable salads served inrestaurants and cafeteria of Duhok city. A total of 200 samples of vegetables namely cucumber, seleq, lettuce, green onion, parsley, tomato, cabbage, carrot, green paper and corgette were collected from retail stores and 55 samples of vegetable salads collected from restaurants and cafeteria.E .coli was isolated in 39 (19.5%) vegetable samples being highest (90%) in parsley and lowest (10%) in tomato but no E. coli O157 was detected in all vegetable samples. E. coli was isolated in 18 (32%) vegetable salads being highest (80%) in the salads of cafeteria and lowest (22.2%) in the salads of restaurants. E. coli O157 was not detected among all isolated E. coli. Conclusion: E. coli O157 is very rare in our area but further studies are required to cover more numbers of samples and to investigate non-O157 shiga-toxin producing E.coli.*

Key words:*E.coli O157, vegetables*

I. Introduction

Escherichia coli O157 is a strain of the Enterohemorrhagic*E.coli* (EHEC) [1] or Shiga Toxin producing *E.coli* (STEC) [2] or verocytotoxigenic*E. coli*(VTEC) [3].

E. coli O157 has been recognized as an important cause of human food borne diseases such as hemolytic uremic syndrome and hemorrhagic colitis [4]. The main reservoirs of this strain are ruminants such as cattle, sheep and goats [5].

E. coli O157 can be transmitted to humans by foods and vegetables contaminated by animal fecal materials.Although undercooked ground beef meat has been identified as a leading food vehicle of *E .coli* O157, fresh raw vegetables are also becoming increasingly important vehicles of food born transmission [6]. Many outbreaks of *E .coli* O157 infections were associated with contaminated leafy lettuce [7], radish sprout [8], alfalfa sprout [9], potatoes[10].

Contamination of vegetables with *E.coli* O157 may occur at different stage from cultivation to transportation[7]. Vegetables grown in soil fertilized by animal manure have a great chance to be contaminated with *E.coli* O157[11]. *E.coli* O157:H7 can enter the lettuce tissue when lettuce seeds are grown in manure fertilized soil[12]. Some vegetables like spinach may be contaminated by manure fertilized soil, or by irrigation with water mixed with sewage or by contaminated surface water irrigation [13].

Vegetables used for preparing salads may be the possible routes of transmission if they had been grown in soil fertilized by human wastes or animal manure. Vegetables grown in soil fertilized by human waste carry different kinds of microorganisms[14]. *E. coli* O157 can be also transmitted from vegetables used for salads by hands [15].

Although few researches were carried out recently on the prevalence of *E.coli* O157 in cattle fecal materials, beef ground meat,drinking waters from wells, diarrheic stools [16] and in sheep and goats milk [17] but there is no data on *E.coli* O157 from raw vegetables consumed in the area, therefore the present study aims to find out the presence of *E.coli* O157 in both raw unwashed vegetables sold at retail vegetable stores as well as in the washed vegetables served as salads in restaurants and cafeteria in Duhok city, Kurdistan Region, Iraq.

II. Materials and Methods

2.1. Sampling

A total of 255 samples were collected from January to November 2013 from retail vegetable stores in which 200 samples from ten different raw vegetables (10 samples for each type) namely cucumber, seleq, lettuce, green onion, parsley, tomato, cabbage, carrot, green paper and corgette, 55 samples of salads served at restaurants and cafeterias of Duhok city. All samples were randomly collected in sterile plastic bags, labeled and transported to the Microbiology laboratory/ department of Biology, Faculty of Science, UniversityofDuhok for analysis.

2.2. Isolation and Identification of *E. coli*

Each sample was homogenized in a sterile electrical blender and 1 gram of the homogenized sample was suspended in 9 ml of sterile physiological saline (0.85% NaCl). Serial dilutions up to 10^{-7}were made and 1 ml of each dilution was plated on both Eosin Methylene Blue (EMB) and Mac Conkey agars. The plates were incubated at 37 Cº for 24 hours. Colonies exhibited metallic sheen on EMB agar and pink color on Mac Conkey agar were subcultured to obtain pure culture. Pure cultures were tested biochemically using API 20 E strips (Bio Merioux, France) to confirm as *E. coli*.

2.3. Identification of *E. coli* O157

All isolated *E. coli* were tested serologically for O157 antibody using slide agglutination test with specific *E.coli* O157 antiserum (Oxoid) according to the manufacture instructions.

III. Results

A total of 39 (19.5%) of *E.coli* were isolated from 200 samples of raw vegetables in which highest percentage (90%) was isolated from parsley, while lowest percentage (10%) was isolated from tomato (Table 1). No *E.coli* O157 detected among all isolated *E.coli* as shown in Table 1.

A total of 18 (32.7%) of *E.coli* were isolated from salads served in restaurants and cafeteria in which 80% from salads served in cafeteria and 22.22% from salads served in restaurants as shown in Table 2. No *E .coli* O157 was detected among all isolated *E.coli* from salads served in both restaurants and cafeteria (Table 2).

Table.1 Number and percentages of *E.coli*and *E. coli*O157 isolated from raw vegetables using latex anti- O157 antibody test

SN	Sample	No.of samples	No. and % of isolated *E.coli*	No. of isolated *E.coli* O157
1	Cucumber	10	3 (30)	0
2	Seleq	10	3 (30)	0
3	Lettuce	10	3 (30)	0
4	Green onion	10	8 (80)	0
5	Parsley	10	9 (90)	0
6	Tomato	10	1 (10)	0
7	Cabbage	10	3 (30)	0
8	Carrot	10	4 (40)	0
9	Green pepper	10	3 (30)	0
10	Corgette	10	2 (20)	0
Total		**200**	**39 (19.5)**	**0**

Table.2 Number and percentages of *E. coli* and *E. coli* O157 isolated from salads using latex anti- O157 antibody test

Source of samples	Total No. of samples	No. and % of *E. coli*	No. and % of *E. coli* O157
Restaurants	45	10 (22.22)	0 (0)
Cafeteria	10	8 (80)	0(0)
Total	55	18 (32.72)	0(0)

IV. Discussion

E.coli O157 is the most studied strains among all other pathogenic strains of *E.coli* because it has been recognized as the leading causes of human food born infections throughout the world with fatal complications such as hemolytic uremic syndrome that ends in renal failure. This strain can be transmitted to humans by direct and indirect methods such as contaminated beef ground meat, water, raw milk, fruit juice and vegetables. It has been recognized that vegetables can be an important vehicles for transmitting *E.coli* to humans. In this study *E.coli*was detected in 19.3% from raw unwashed vegetables which was lower than those recorded by Enabulele and Uraih (2009) from Nigeria [18]. These low results can be attributed to the samples size, geographical area and agricultural methods for vegetable production (open farm verses plastic houses).*E.coli* is the normal flora in the digestive tract of both humans and animals and could contaminate the vegetables from different source either from animal manures used as soil fertilizer or through contaminated transportation vehicles. The percentage of *E.coli* was 32% from washed vegetables served as salads in restaurants and cafeteria of Duhok city in which high percentage 80% was from salads served in cafeteria and lower percentage 22.22% from

salads served in restaurants. This high percentage of *E. coli* isolated from washed salads compared to raw unwashed vegetables may be due to the hands of food handlers or containers.

No *E.coli* O157 was detected in both raw unwashed vegetables and salads. These results were similar to that reordered by Enabulele and Uraih [18] who did not detect *E.coli* O157 among all isolated *E.coli* form vegetables, but disagreed with the results of Sharif and Arafa[19] who found *E.coli* O157 in 11.7% of studied vegetables and Abong [20] who found prevalence of *E.coli* O157 ranged from 0% to 33% in onions and cabbage respectively.

These differences in the results could be contributed to many factors such as sample size, methodology used for identification, geographical variations, seasonal variation and hygienic precautions. Absence of *E.coli* O157 among isolated *E.coli* does not rule out other non-O157 shiga-toxin *E.coli* (STEC) which may be the main causes of food poisoning in our area and require investigation. Johnson and co-workers [21]reportedthat human are more exposed to non-O157 shiga-toxin *E.coli* from contaminated foods and environmental sources than to *E.coli* O157.

According to the current results and results of the previous studies carried out in the area, *E.coli* O157 is very rare and warrant studies focusing on non-O157 STEC.

V. Conclusions

1- *E. coli O157* is very rare in our area.

2- Investigation of non O157 shiga toxin producing *E. coli* (STEC) is required.

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